A UNIVERSAL PROBE SPECIFIC FOR (PER)CHLORATE-REDUCING BACTERIA

Susan O'Connor, John D. Coates, Susan Rogers, and John Martinko

Department of Microbiology,
Southern Illinois University, Carbondale, IL 62901
Corresponding author tel: (618) 453-6132, e-mail: jcoates@micro.siu.edu

Oxyanions of chlorine such as perchlorate (ClO_4^-), chlorate (ClO_3^-), chlorite (ClO_2^-), and chlorine dioxide (ClO_2) have a wide array of industrial applications including use as bleaching agents by the paper and pulp industry, as disinfectants and defoliants by the agricultural industry, and as components of explosives and rocket propellants by the aerospace and defense industries. Chloro-oxyanions are generally not formed naturally and have only been introduced into the environment through anthropogenic sources in the last 100 years. Recently, much attention has focussed on the widespread contamination of ground and surface waters by perchlorate, however, this represents only one aspect of the contamination problems associated with these compounds. Chloro-oxyanions can be formed in the environment as a result of ozonation of drinking waters which have been treated with chlorine. The chemistry of these compounds can be quite complex depending on the species present. In water chlorine dioxide and hypochlorite decompose abiotically into chlorite, chlorate, and chloride. Chlorite can be dismutated into chlorate and chloride. The widespread use of these compounds, frequent improper disposal, and recent arms reduction treaties and aging missile inventories of the US Air Force has led to increased interest in the biological treatment of chlorooxo compound contaminants and wastes.

Recent studies in our lab have demonstrated the ubiquity and diversity of (per)chlorate-reducing bacteria (ClRB) which couple growth under anaerobic conditions to the reduction of chlorate or perchlorate ((per)chlorate) to innocuous chloride. We identified two taxonomic groups, the *Dechlorimonas* and the *Dechlorisoma* groups, which represent the dominant ClRB in the environment. As part of these studies we demonstrated that chlorite dismutation is a central step in the reductive pathway of (per)chlorate that is common to all (per)chlorate-reducing bacteria. Chlorite dismutation is mediated by a single enzyme, chlorite dismutase (CD) that is specific to the ClRB. Initial studies on CD suggested that this enzyme is highly conserved amongst the ClRB, regardless of their phylogenetic affiliation. As such this enzyme makes an ideal target for a probe specific for these organisms.

Chlorite dismutase enzyme was purified to homogeneity from *Dechlorimonas agitatus* strain CKB. Polyclonal antibodies against CD were raised in New Zealand type rabbits. The immunogen was prepared by mixing together equal amounts of CD (1mg/mL) and complete Freunds adjuvant, and the rabbits were immunized subcutaneously. The secondary immunizations were prepared with incomplete Freunds adjuvant. Test bleeds were made 10-14 days after immunization, from the marginal ear vein of the rabbit.

The antigen binding activity of the antisera was assessed using an ELISA based upon the capture of the CD antibodies by immobilized CD enzyme with subsequent visualization by goat

anti-rabbit IgG linked to alkaline phosphatase. The titer values obtained with purified CD (3000 - 5000) indicated that the anitsera had a high affinity for the CD enzyme (data not shown).

The antisera was used as an immunoprobe against different isolates of ClRB representing the alpha, beta , and gamma subclasses of the Proteobacteria. Dot-blot and ELISA techniques were employed with both whole cell suspensions and cell lysates of these isolates. Cell lysates of closely related organisms to the ClRB which do not grow by dissimilatory (per)chlorate reduction were also investigated.

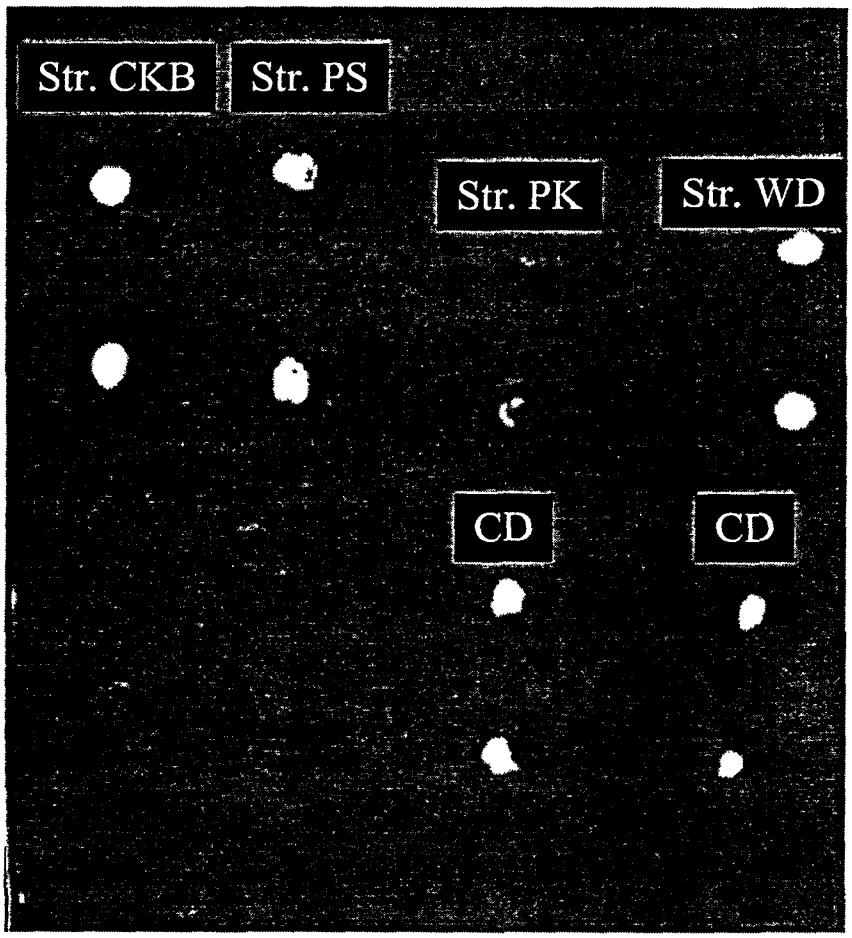

Figure 1: Dotblot of cell lysates prepared from active cultures of (per)chlorate-reducing bacteria and the purified chlorite dismutase enzyme. Str. CKB:- *Dechlorimonas agitatus*; str. PS:- *Dechlorisoma suillus*; str. PK:- *Pseudomonas* strain PK; str. WD:- *Dechlorospirillum anomalous* ; CD:- purified chlorite dismutase from *D. agitatus* strain CKB.

All the ClRB cell lysates tested positive regardless of phylogenetic affiliation (Fig. 1). Interestingly, the titer values obtained for each of the ClRB cell lysates was different suggesting that there may be some minor differences in the CD protein produced in these organisms (data not shown). No reactions were observed with cell lysates of the closely related organisms to the

ClRB which could not grow by (per)chlorate reduction. Furthermore, no reactions were observed with controls prepared from yeast extract or bovine serum albumin further supporting the specificity of this antisera for CD. In contrast, in studies on whole cell the antisera effectively bound to the cell surface of *D. agitatus* and other members of the *Dechlorimonas* group, however, the antisera did not react with whole cells from the *Dechlorisoma* group or any of the other ClRB isolates tested (Fig. 2). As the CD enzyme in cell lysates does react with the antisera, these results indicate that the CD enzyme is expressed differently in the various ClRB tested.

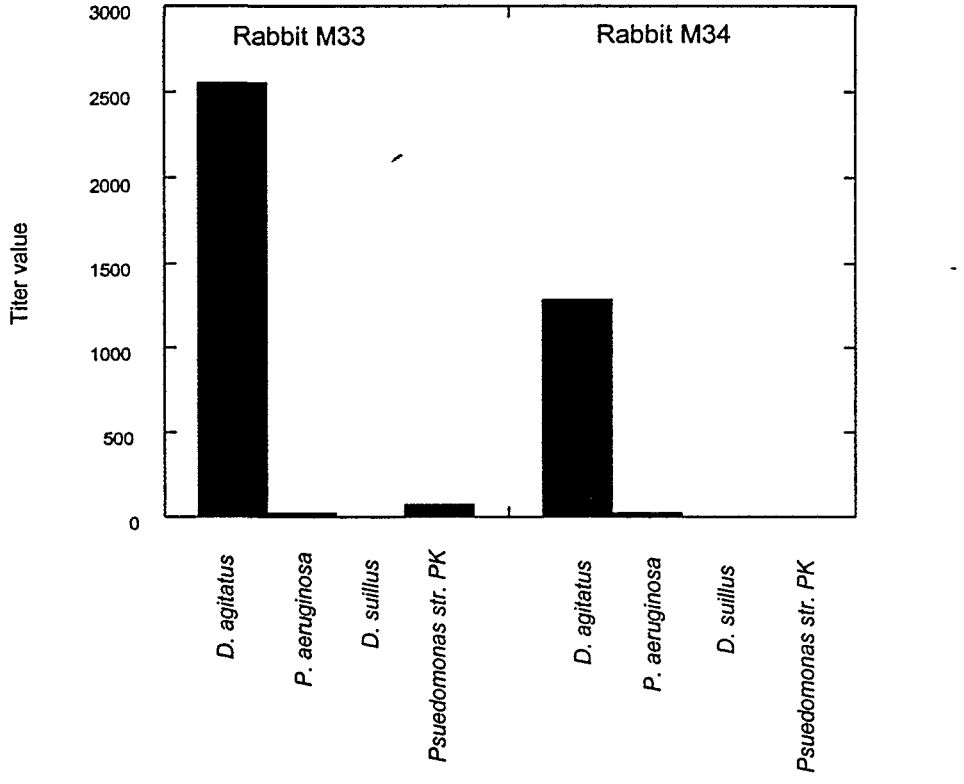

Figure 2: Immunoprobe titers obtained against whole cells of the (per)chlorate-reducing organisms*Dechlorimonas agitatus, Dechlorisoma suillus, Pseudomonas* strain PK, and a control organism, *Psuedomonas aeruginosa*which cannot grow by (per)chlorate reduction and does not contain chlorite dismutase. The results demonstrate that the raised antibodies only bind to the cell surface of *D. agitatus* type organisms which suggests that the chlorite dismutase is expressed differently in the different organisms.

These studies have resulted in the development of a highly specific immunoprobe which can be used to assess dissimilatory perchlorate-reducing populations in environmental samples regardless of their phylogenetic affiliations. The probe is based on the commonality of the chlorite dismutase enzyme in ClRB and in the case of members of the *Dechlorimonas* group may be used to rapidly identify and enumerate whole cells.